

EVALUATION OF FUNGICIDES AGAINST *PHYTOPHTHORA* AND *FUSARIUM* (root rot spp.) OF CITRUS ROOTSTOCKS SEEDLINGS

Shakila Yasmeen^{1,*}, Muhammad Mumtaz Khan¹, Saeed Ahmad¹ Mazhar Abbas¹, Bushra Sadia² and Muhammad Azam²

¹Institute of Horticultural Sciences, University of Agriculture, Faisalabad; ²CABB, University of Agriculture, Faisalabad

*Corresponding author's e-mail: shakeela227@gmail.com

Citrus is one of the most important fruit crop in the world and is usually grown through grafting technique. Rootstock is one of the significant part in grafted plants and has crucial effect on production, including yield, fruit quality, tree size, tolerance to salts and diseases, and scion compatibility. Citrus is susceptible to several fungal pathogens causing incalculable losses to the crop. Among all soil-borne fungal pathogens, *Phytophthora* and *Fusarium* cause the most severe damage to the nursery or orchards plants. This research was planned to evaluate the effectiveness of fungicides as soil drenching and root dipping to control *Phytophthora* and *Fusarium* attacking citrus rootstock seedlings at the nursery stage. Different physiological and morphological parameters were studied in the infected plants and data were compared with that of control. The data were recorded and compared concerning rootstock seed and seedling response using standard measures and statistical analysis. The results showed that plants inoculated with *Phytophthora* and *Fusarium* root rot spp. when treated with Aliette and Ridomil Gold showed maximum root shoot ratio, fresh dry weight ratio, photosynthetic rate, stomatal conductance, water potential and transpiration rate as compared to untreated plants. The results also depicted that plants treated with Aliette and Ridomil Gold through soil drenching have maximum root shoot ratio, fresh dry weight ratio, photosynthetic rate, stomatal conductance and transpiration rate as compared to root dipped plants.

Keywords: Fungal diseases, pathogens, root rot, nursery plants.

INTRODUCTION

Phytophthora and *Fusarium* are the most devastating pathogens for citrus plants, especially at the seedling or nursery stage (Hussain and Abid, 2011). *Phytophthora* species cause severe citrus diseases worldwide (Savita *et al.*, 2012). Root rot caused by *Phytophthora nicotianae* (syn. P.n.var. *parasitica*) and *P. citrophthora* is one of the significant soil-borne diseases of citrus in all growing areas of the world (Ippolito *et al.*, 2004). The most severe citrus diseases caused by *Phytophthora* are foot rot and gummosis. *Fusarium* is also considered a severe pathogen attacking citrus plants' roots (El-Nouby, 2002). *Fusarium* can induce symptomless infection in citrus roots but, under stress conditions, it causes severe dry root rot, which usually does not seep gum, while the most conspicuous symptom above ground is a fatal collapse of the tree; leaves wilt suddenly and in a few days dry up, remaining attached on the tree (Spina *et al.*, 2008). The primary means by which these pathogens spread in the orchards is the use of infected seeds and infested nursery stock. While water, air and contaminated soil also

spread the pathogen from one place to another (Landis *et al.*, 1990). So, it is vital to use disease-free nursery plants to avoid contamination in the orchard.

The objective of this experiment was to evaluate the effectiveness of fungicides as soil drenching and root dipping to control *Phytophthora* and *Fusarium* spp. attacking citrus rootstock at the seedling stage. Data was collected regarding no. of infected and non-infected plants, Stomatal conductance, transpiration rate, photosynthetic rate, leaf water potential, root shoot length ratio, root shoot fresh, and dry weight ratio. The data were recorded and compared concerning rootstock response using standard measures and statistical analysis.

MATERIALS AND METHODS

This research was conducted in Citrus Nursery Sanitation Laboratory, Institute of Horticultural Sciences in cooperation with the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. 6-month-old Rough

Yasmeen, S., M.M. Khan, S. Ahmad, M. Abbas, B. Sadia and M. Azam. 2021. Evaluation of fungicides against *phytophthora* and *fusarium* (root rot spp.) of citrus rootstocks seedlings. J. Glob. Innov. Agric. Sci.9:145-154.

[Received 13 Aug 2021; Accepted Oct 2021; Published (online) 30 Dec 2021]



Attribution 4.0 International (CC BY 4.0)

lemon rootstock seedlings were treated with both fungicides by two methods (drenching and root dipping).

Plant Material: Six months old uniform-sized Rough lemon (*Citrus jambhiri*) rootstock seedlings were selected from "Citrus Nursery of Certified Plants" Institute of Horticultural Sciences, University of Agriculture, Faisalabad.

Preparation of Inoculum: *Phytophthora citrophthora* and *Fusarium solani* used in this study were isolated from specimens having symptoms of decline suspected due to fungal diseases, collected Experimental fruit garden square No 9, University of Agriculture Faisalabad. The culture was prepared through the spore suspension method at 10^5 spores/ml (Timmer, 1982).

Application of fungicides

Soil Drenching: Selected Rough lemon rootstock seedlings were planted in pots containing planting media composed of silt and sand at 1:1. The pots were inoculated with seven days old cultures of *Phytophthora citrophthora* and *Fusarium solani* using the spore suspension method before planting the seedlings.

Aliette

Control(No fungicide)
D₁1.0g dissolved in 1L water
D₂2.0g dissolved in 1L water
D₃3.0g dissolved in 1L water

Ridomil Gold

Control(No fungicide)
D₁7.0g dissolved in 1L water
D₂8.0g dissolved in 1L water
D₃9.5g dissolved in 1L water

Root Dipping: Six-month-old uniform Rough lemon seedlings were dipped into fungicide solution for one minute and then planted in pots containing the planting media. The pots were inoculated with seven days old cultures of *Phytophthora citrophthora* and *Fusarium solani* using the spore suspension method before planting the seedlings.

Aliette

Control(No fungicide)
D₁1.0g dissolved in 1L water
D₂2.0g dissolved in 1L water
D₃3.0g dissolved in 1L water

Ridomil Gold

Control(No fungicide)
D₁7.0g dissolved in 1L water
D₂8.0g dissolved in 1L water
D₃9.5g dissolved in 1L water

Evaluation: Data regarding no. of infected and non-infected plants, stomatal conductance, transpiration rate, photosynthetic rate, leaf water potential, root shoot length ratio and root shoot fresh and dry weight ratio was collected and compared with control.

RESULTS

Effect of Aliette soil drenching and root dipping on Root shoot ratio of Rough lemon seedlings inoculated with *Fusarium*: The results showed that fungicide-treated seedlings have a maximum root shoot ratio compared to control. The results followed the observation of Einhellig (1995). The results also showed that drenched soil seedlings have the highest root shoot ratio 0.891, 0.831 and 0.784 at doses 1g/L, 2g/L and 3g/L, respectively, compared to root dipped and control (Fig.1).

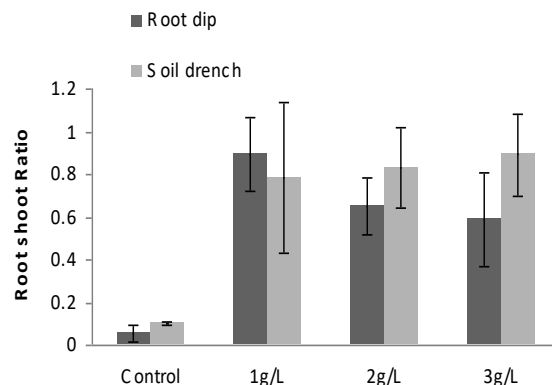


Figure 1. Effect of Aliette soil drenching and root dipping on root shoot ratio of rough lemonseedlings Inoculated with *Fusarium*.

Effect of Aliette soil drenching and root dipping on fresh/dry weight ratio of rough lemon seedlings inoculated with *Fusarium*: The results confirmed that untreated seedlings have a minimum Fresh/dry weight ratio compared to treated seedlings (Fig.2). The same results were observed by Rajput *et al.* (2008) that there was a reduction in plant growth and weight of plants injected with *Fusarium*. The results also showed that Aliette soil drenched seedlings have a maximum fresh/dry weight ratio compared to root dipped and control. Le Roux *et al.* (1998) also reported that citrus trees soil drenched with fungicide have better growth than untreated. The higher fresh/dry weight ratio (1.735 mg) was gained by drenched soil seedlings treated with 2 g/L followed by 1 g/L and 3 g/L with 1.563 mg and 1.391 mg, respectively.

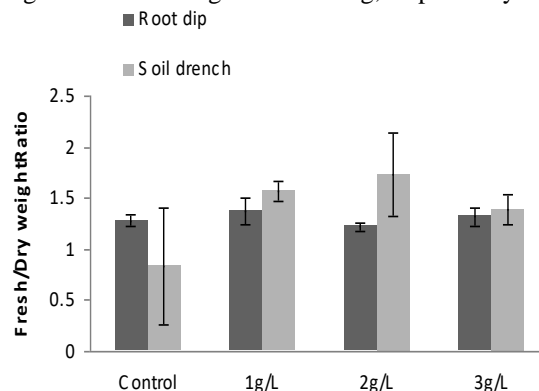


Figure 2. Effect of Aliette soil drenching and root dipping on fresh/dry weight ratio of rough lemon seedlings inoculated with *Fusarium*.

Effect of Aliette soil drenching and root dipping on photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of rough lemon seedlings inoculated with *Fusarium*: The result showed that the Photosynthetic rate of treated seedlings was maximum compared to control. Similar findings were documented by Jaleel *et al.* (2007) that plants treated with fungicide against

root rot fungi have a maximum photosynthetic rate compared to control. The results also showed that the photosynthetic rate of soil-drenched seedlings was also maximum compared to the root dipped. The soil-drenched seedlings have the highest photosynthetic rate ($1.42 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at dose 1g/L (Fig.3).

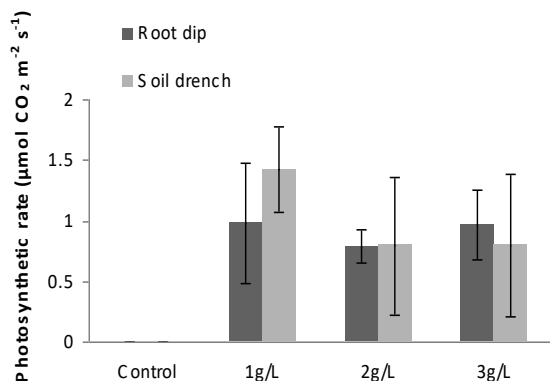


Figure 3. Effect of Aliette soil drenching and root dipping on photosynthetic rate of rough lemon seedlings inoculated with *Fusarium*.

Effect of Aliette soil drenching and root dipping on Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of Rough lemon seedlings inoculated with *Fusarium*: Rough lemon seedlings treated with Aliette showed maximum transpiration rate compared to control. The results also show that soil-drenched seedlings showed maximum transpiration rate over root-dipped seedlings (Walker, 1987). The soil-drenched seedlings treated with 1g/L Aliette have the higher transpiration rate ($1.603 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), followed by 2g/L and 3g/L with 1.587 $0.680 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ respectively (Fig.4).

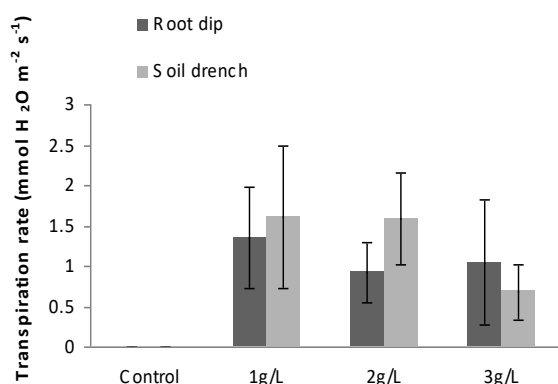


Figure 4. Effect of Aliette soil drenching and root dipping on transpiration rate of rough lemon seedlings inoculated with *Fusarium*.

Effect of Aliette soil drenching and root dipping on Stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$) of Rough lemon seedlings inoculated with *Fusarium*: Seedlings treated with fungicide have maximum stomatal conductance compared to untreated.

The results are in accordance with Gopi *et al.* (2008) that plants treated with fungicide have an increased number of stomatal cells per unit area and increased stomatal conductance compared to untreated plants. The Rough lemon seedlings treated with Aliette through soil drenching have the highest stomatal conductance 22.33, 21.00 and 13.33 $\text{mmol m}^{-2} \text{ s}^{-1}$ at doses 1g/L, 2g/L and 3g/L, respectively, as compared to root dipped and control (Fig. 5).

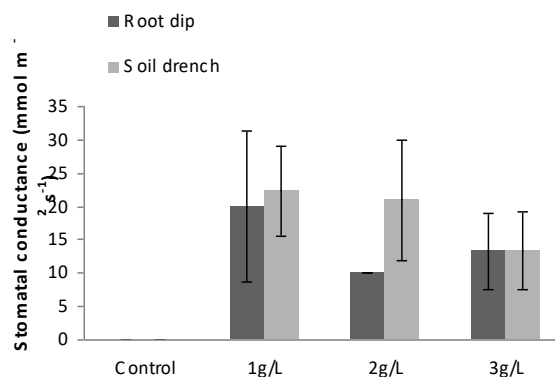


Figure 5. Effect of Aliette soil drenching and root dipping on stomatal conductance of rough lemon seedlings inoculated with *Fusarium*.

Effect of Aliette soil drenching and root dipping on water potential (MPa) of rough lemon seedlings inoculated with *Fusarium*: The results showed that seedlings treated with fungicide have more water potential than untreated. The results are the same as Maurel *et al.* (2001) reported. The results also showed that soil-drenched seedlings have more water potential than root dipped. The seedlings treated through soil drenching have the highest water potential 1.697, 0.927 and 0.260 MPa at doses 3g/L, 1g/L and 2g/L, respectively (Fig.6).

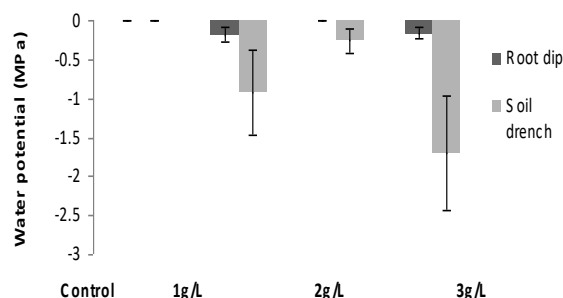


Figure 6. Effect of Aliette soil drenching and root dipping on leaf water potential of rough lemon seedlings inoculated with *Fusarium*.

Effect of Aliette soil drenching and root dipping on root shoot ratio of rough lemon seedlings inoculated with *Phytophthora*: The results showed that the maximum root shoot ratio was obtained by the Aliette root dipped and soil drenched seedlings compared to control (Fig.7). The same

findings were obtained by Marias and Hattingh (1983) that by the application of Foestyl-Al and Metalaxyl against *Phytophthora*, there was a significant increase in root shoot length. The highest root shoot (0.83) was gained by Aliette soil drenched seedlings treated with 2g/L.

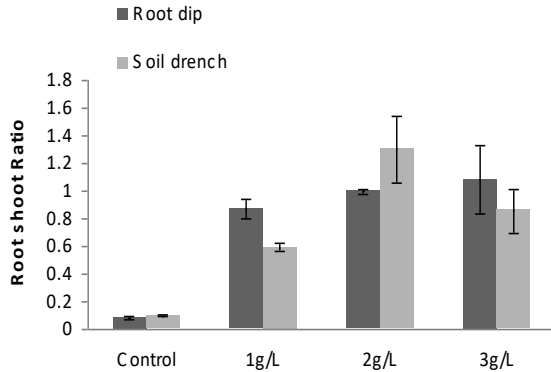


Figure 7. Effect of Aliette soil drenching and root dipping on root shoot ratio of rough lemon seedlings inoculated with *Phytophthora*.

Effect of Aliette soil drenching and root dipping in fresh/dry weight ratio of rough lemon seedlings inoculated with *Phytophthora*: The results showed that treated seedlings have a maximum mean fresh/dry weight ratio than untreated (Fig. 8). The results also showed that soil-drenched Rough lemon seedlings have a maximum mean fresh/ dry weight ratio than root dipped and untreated. Similar results were obtained by Fenn and Coffey (1983) that seedlings soil drenched with Foestyl-Al against *Phytophthora* have maximum mean fresh/dry weight ratio. The highest mean fresh/dry weight ratio (2.287) was gained by soil-drenched seedlings treated with 1g/L.

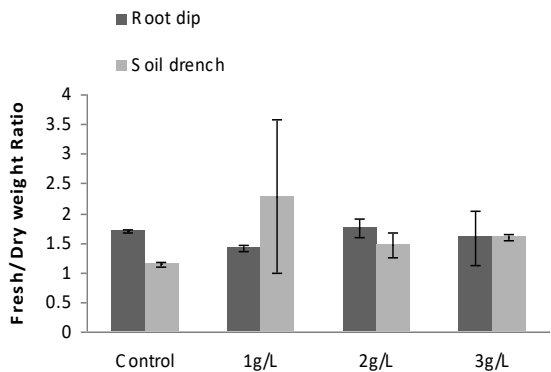


Figure 8. Effect of Aliette soil drenching and root dipping on fresh/dry weight rough lemon seedlings inoculated with *Phytophthora*.

Effect of Aliette soil drenching and root dipping on Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of rough lemon seedlings inoculated with *Phytophthora*: The result showed

that the photosynthetic rate of soil drenched and root dipped seedlings was maximum compared to control. Similar results were documented by Jaleel *et al.* (2007) that plants treated with fungicide against root rot fungi have a maximum photosynthetic rate compared to control. The results also showed that soil-drenched treated seedlings have more photosynthetic than root dipped and control. The highest photosynthetic rate ($5.433 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was gained by soil drenched seedlings treated at 3 g/L followed by 2g/L and 1 g/L (Fig. 9).

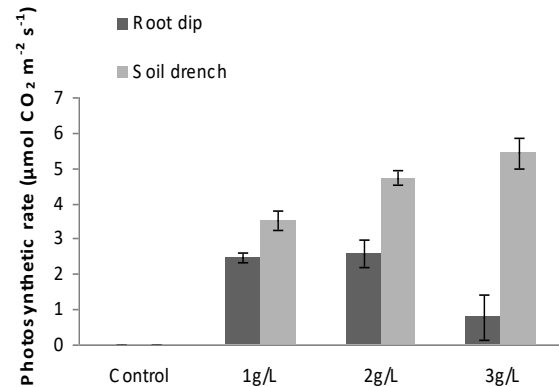


Figure 9. Effect of Aliette soil drenching and root dipping on photosynthetic of rough lemonseedlings inoculated with *Phytophthora*.

Effect of Aliette soil drenching and root dipping on Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of rough lemon seedlings inoculated with *Phytophthora*: The results showed that untreated plants inoculated with *Phytophthora* were not in such a condition to regulate the important physical process such as transpiration compared to soil drenched and root dipped. The results are in accordance with Fleischmann *et al.* (2002) also found that a few days after inoculation, photosynthesis and transpiration of seedlings infected with either *P. citricola* or *P. cambivora* were strongly reduced. The results also showed that seedlings soil drenched have maximum transpiration rate compared to root dipped seedlings (Fig. 10).

Effect of Aliette soil drenching and root dipping on stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$) of rough lemon seedlings inoculated with *Phytophthora*: The treated seedlings have better stomatal conductance compared to untreated seedlings inoculated with *Phytophthora*. The results follow Maurelet *et al.* (2001) that inoculation of *Phytophthora* decreased stomatal conductance and leaf water potential. The results also showed that drenched soil plants have maximum stomatal conductance compared to root dipped (Fig.11). The results suggest that the soil drenching method can enhance the stomatal conductance of seedlings under *Phytophthora* infection conditions (Gopi *et al.*, 2008).

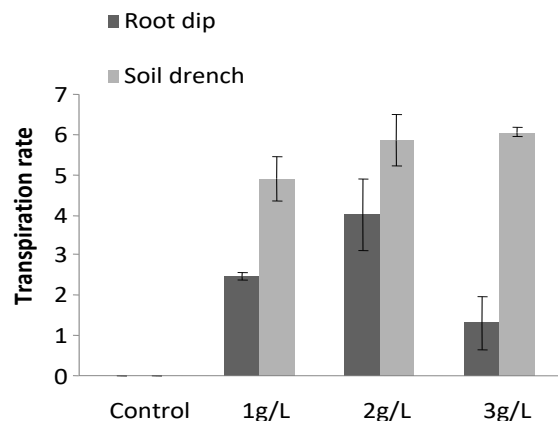


Figure 10. Effect of Aliette soil drenching and root dipping on transpiration rate of rough lemon seedlings inoculated with *Phytophthora*.

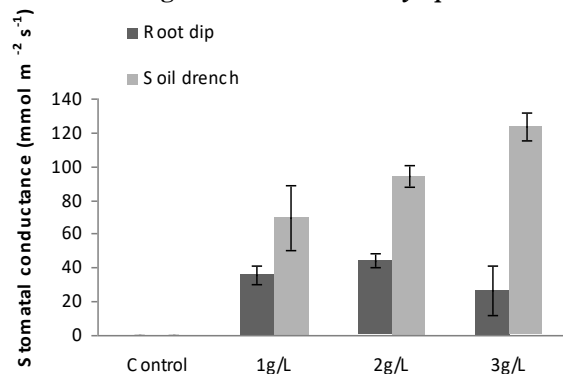


Figure 11. Effect of Aliette soil drenching and root dipping on stomatal conductance of rough lemon seedlings inoculated with *Phytophthora*.

Effect of Aliette soil drenching and root dipping on Water potential (MPa) of Rough lemon seedlings inoculated with *Phytophthora*: Results showed that treated seedlings have more water potential than untreated seedlings (Fig.12). The results are similar to the findings of Dawson and Weste (1992), confirming that *Phytophthora* infection was associated with reduced relative water content and leaf water potential. The results also showed that drenched soil seedlings have maximum Leaf water potential compared to root dipped and control. The soil-drenched seedlings gave the highest leaf water potential (2.628 MPa) at 3 g/L followed by 1.242 and 0.35 MPa at 2 g/L and 1 g/L.

Effect of Ridomil Gold soil drenching and root dipping on Root shoot ratio of rough lemon seedlings inoculated with *Fusarium*: The results showed a significant interaction between fungicide application methods and treatments, as untreated seedlings have the lower root shoot ratio compared to treated seedlings (Fig.13). Einhellig (1995) documented similar results. The results also showed that soil-drenched Rough lemon seedlings have a maximum root shoot ratio than

root dipped and control. The soil-drenched seedlings had the highest root shoot ratio (2.919) when Ridomil Gold was applied 8g/L.

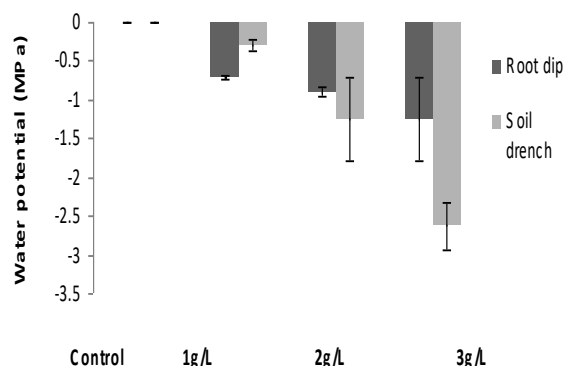


Figure 12. Effect of Aliette soil drenching and root dipping on water potential of rough lemon seedlings inoculated with *Phytophthora*.

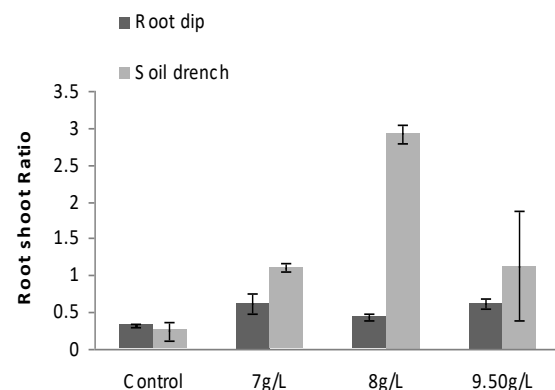


Figure 13. Effect of Ridomil Gold soil drenching and root dipping on root shoot ratio of rough lemon seedlings inoculated with *Fusarium*.

Effect of Ridomil Gold soil drenching and root dipping on Fresh/Dry weight ratio of Rough lemon seedlings inoculated with *Fusarium*: The results showed that treated seedlings have a better fresh/dry weight than untreated seedlings (Fig.14). Similar results were observed by Gundy and Tsao (1963) that there was a decrease in citrus seedlings inoculated with *Fusarium*. The results also showed that soil-drenched treated seedlings inoculated with *Fusarium* gained maximum fresh/dry weight ratio compared to root dipped and untreated. The fresh/dry weight ratio of soil drenched is higher (3.067) at dose 8g/L followed by 7g/L and 9.50g/L.

Effect of Ridomil Gold soil drenching and root dipping on Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of Rough lemon seedlings inoculated with *Fusarium*: The results showed that treated seedlings have a maximum photosynthetic rate compared to untreated (Fig.15). The results are similar as Siddiqui and Zman (2006) documented. The results also

showed that drenched soil seedlings have a maximum photosynthetic rate than root dipped and control. The results also showed that root dipped and untreated seedlings were failed to carry on photosynthesis reaction as the pathogen greatly infected the vascular system. The results are similar to the conclusion of Ploetz and Schaffer (1992) that plants infected with fungal pathogens had a reduced photosynthetic rate.

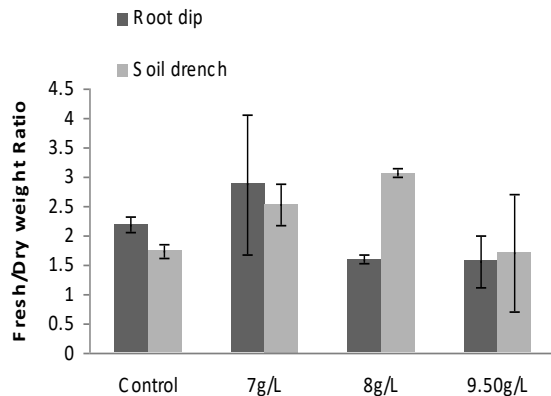


Figure 14. Effect of Ridomil Gold soil drenching and root dipping on fresh/dry rough lemon inoculated with *Fusarium*.

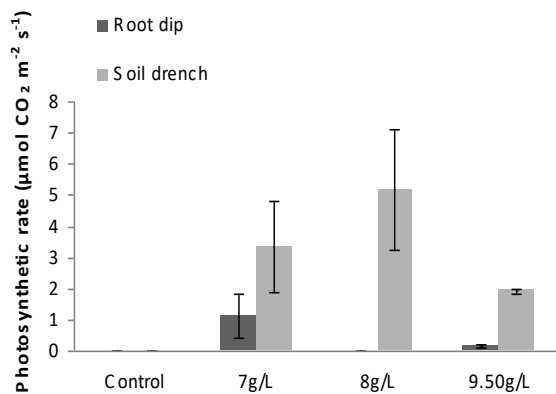


Figure 15. Effect of Ridomil Gold soil drenching and root dipping on photosynthetic rate rough lemon seedlings inoculated with *Fusarium*.

Effect of Ridomil Gold soil drenching and root dipping on Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of rough lemon inoculated with *Fusarium*: The results showed that Ridomil Gold treated seedlings inoculated with *Fusarium* have a maximum transpiration rate than untreated seedlings. The results are similar to the findings of Walker *et al.* (1987). The results also showed that soil-drenched seedlings transpire efficiently compared to root-dipped and untreated seedlings. The soil-drenched seedlings have the highest transpiration rate ($4.337 \text{ mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) treated with 8g/L (Fig.16).

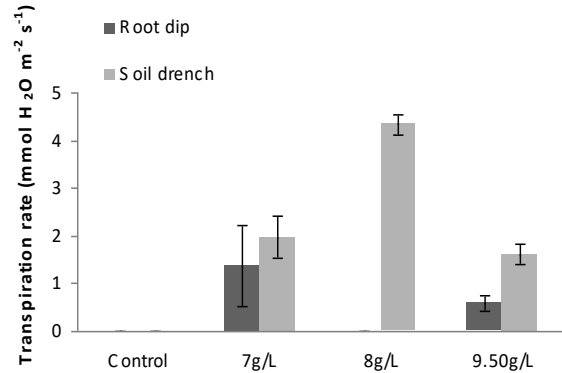


Figure 16. Effect of Ridomil Gold soil drenching and root dipping on transpiration rate of rough lemon seedlings inoculated with *Fusarium*.

Effect of Ridomil Gold soil drenching and root dipping on Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) of Rough lemon seedlings inoculated with *Fusarium*: The results showed that stomatal conductance of untreated, *Fusarium* inoculated seedlings is nil compared to treated. Similar results were observed by Nemecet *al.* (1986) that seedlings inoculated with *Fusarium* had decreased leaf stomatal conductance. The results also showed that soil-drenched Rough lemon seedlings have the highest stomatal conductance compared to root dipped and untreated seedlings (Fig.17). The results are in accordance with (Gopi *et al.*, 2008).

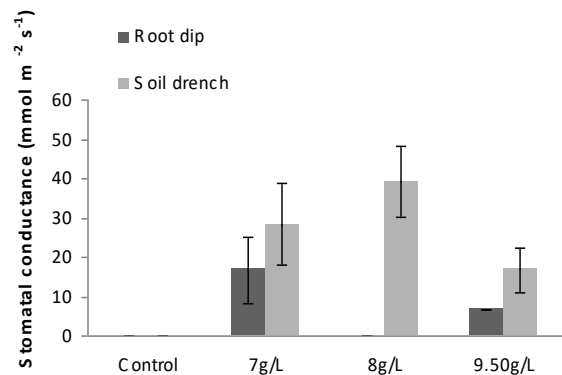


Figure 17. Effect of Ridomil Gold soil drenching and root dipping on stomatal conductance of rough lemon seedlings inoculated with *Fusarium*.

Effect of Ridomil Gold soil drenching and root dipping on Leaf water potential (MPa) of rough lemon seedlings inoculated with *Fusarium*: The results showed that seedlings treated with fungicide showed maximum water potential irrespective of application method against *Fusarium* compared to untreated. Nemecet *al.* (1986) also observed that plants inoculated with *Fusarium* have lower leaf water potential, lower water content, and higher leaf osmotic values than healthy plants. The results also showed that soil-drenched seedlings have more water potential than root-

dipped and untreated seedlings. The soil-drenched seedlings treated with 8g/L have the highest water potential, 0.887 MPa (Fig.18).

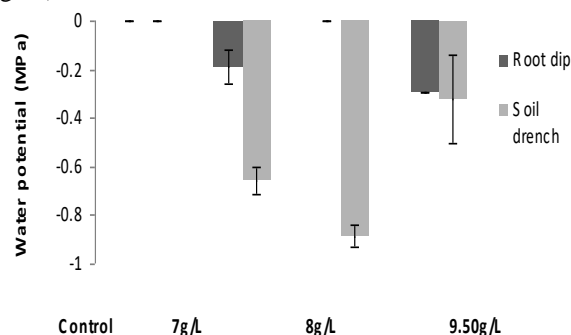


Figure 18. Effect of Ridomil Gold soil drenching and root dipping on leaf water potential of rough lemon seedlings inoculated with *Fusarium*.

Effect of Ridomil Gold soil drenching and root dipping on Root shoot ratio of rough lemon seedlings inoculated with *Phytophthora*: The results showed that treated seedlings have a maximum root shoot ratio compared to untreated. The results also showed that drenched soil seedlings have a maximum root shoot ratio compared to root dipped and untreated. The same results were documented by Walker (1987) that the application of metalaxyl through soil drenching improves plant growth compared to any other application method and untreated plants. The mean root shoot ratio of soil-drenched seedlings increases as fungicide concentration increases (Fig.19). The soil-drenched seedlings treated with 8g/L have the highest root shoot ratio (2.109).

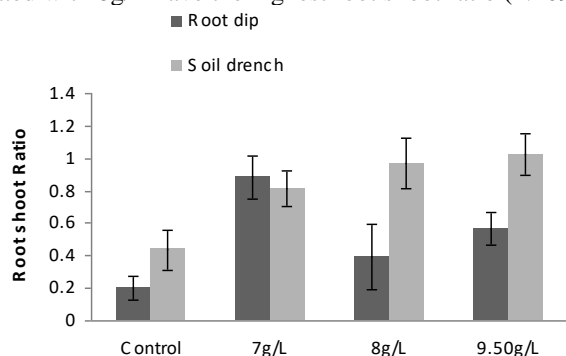


Figure 19. Effect of Ridomil Gold soil drenching and root dipping on root shoot ratio of rough lemon seedlings inoculated with *Phytophthora*.

Effect of Ridomil Gold soil drenching and root dipping on Fresh/Dry weight ratio of rough lemon seedlings inoculated with *Phytophthora*: The results are significant at a 5% significance level (Appendix XVI & XVII). The results showed that treated seedlings have the highest fresh/dry weight compared to untreated seedlings (Fig.20). Soil-drenched seedlings gained the highest mean fresh/dry weight

(2.109) at 9.50 g/L. The results are in accordance with Snyman and Kotze, (1984) that Seedlings treated with metalaxyl drenches had a significantly higher weight compared to untreated seedlings.

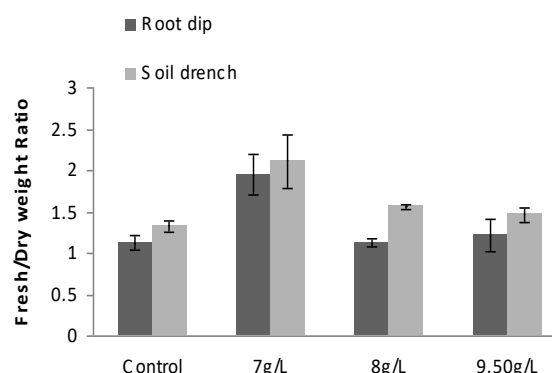


Figure 20. Effect of Ridomil Gold soil drenching and root dipping on fresh/dry weight ratio rough lemon seedlings inoculated with *Phytophthora*.

Effect of Ridomil Gold soil drenching and root dipping on Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of rough lemon seedlings inoculated with *Phytophthora*: The results showed that plants treated with fungicide have a maximum photosynthetic rate compared to untreated (Fig.21). The same results were observed by Jaleel *et al.* (2007) that by the application of fungicide, there was an increased photosynthetic rate compared to control. Plants treated with fungicide have thickened leaves, epidermis, cuticle, plastid and spongy layers. The results also showed that soil-drenched Rough lemon seedlings have the higher photosynthetic rate ($2.237 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at 9.50g/L followed by $2.223 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ plants treated with 8g/L compared to root dipped.

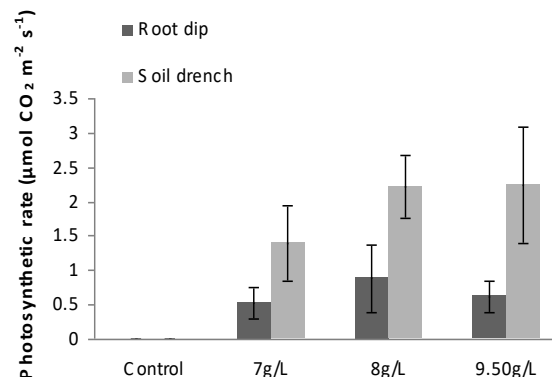


Figure 21. Effect of Ridomil Gold soil drenching and root dipping on photosynthetic rate of rough lemon seedlings inoculated with *Phytophthora*

Effect of Ridomil Gold soil drenching and root dipping on Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of rough lemon seedlings inoculated with *Phytophthora*: The results are

significant at a 5% significance level (Appendix XV & XVI). The results showed that untreated seedlings failed to continue the transpiration process compared to soil drenched and root dipped seedlings (Fig.22). This means that untreated seedlings infected with *Phytophthora* could not maintain the physiological processes. Similar results were obtained by Dawson and Weste (1989) that citrus plants infected with *Phytophthora* have no gas exchange. The results also showed that soil-drenched seedlings have a maximum transpiration rate compared to untreated root dipped. The soil drenched seedlings showed the higher transpiration rate ($2.717 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) when treated with 8g/L followed by 7g/L and 9.50g/L with 2.547 and $2.000 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, respectively.

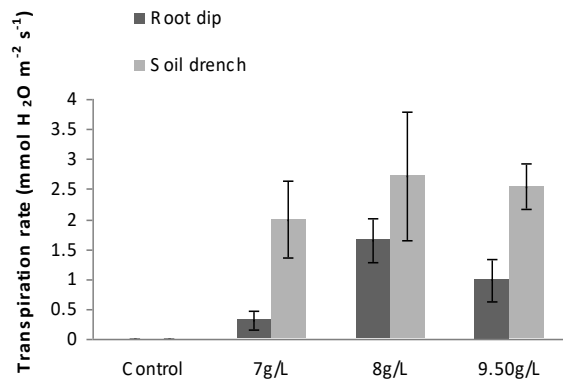


Figure 22. Effect of Ridomil Gold soil drenching and root dipping on transpiration rate of rough lemon seedlings inoculated with *Phytophthora*.

Effect of Ridomil Gold soil drenching and root dipping on stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$) of rough lemon seedlings inoculated with *Phytophthora*: The results showed that seedlings treated with fungicide had better stomatal conductance than untreated (Fig.23). The results are in accordance with Gopi *et al.* (2008) that plants treated with fungicide have an increased number of stomatal cells per unit area and increased stomatal conductance compared to untreated plants. The results also showed that soil-drenched seedlings have the highest stomatal conductance, i.e., $13.333 \text{ mmol m}^{-2} \text{ s}^{-1}$ at the highest fungicide dose 9.50g/L, compared to root dipped and untreated seedlings.

Effect of Ridomil Gold soil drenching and root dipping on Leaf water potential (MPa) of rough lemon seedlings inoculated with *Phytophthora*: The results showed that seedlings treated with fungicide have maximum ability to retain the water compared to root dipped and untreated seedlings (Fig.24). The results proved that soil drenching is an efficient fungicidal application method against *Phytophthora*. The results also showed that seedlings treated through root dipping and untreated are entirely failed to absorb the water from the soil as pathogen greatly injured the plant root system. The results are similar as Bower (1979)

documented that fungal root rot-affected trees could not absorb sufficient moisture from the soil due to root damage.

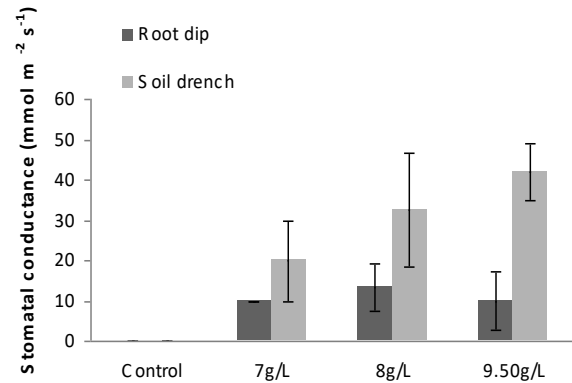


Figure 23. Effect of Ridomil Gold soil drenching and root dipping on stomatal conductance of rough lemon seedlings inoculated with *Phytophthora*.

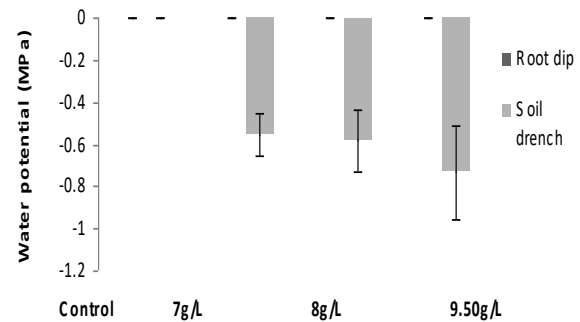


Figure 24. Effect of Ridomil Gold soil drenching and root dipping on leaf water potential of rough lemon seedlings inoculated with *Phytophthora*.

DISCUSSION

Citrus is grown on grafted trees and rootstock is the major part of the grafted trees. It has a considerable effect on all plant characteristics, including yield, fruit quality, tree size, tolerance to salts and diseases (Ahmad *et al.*, 2010). Many rootstocks are susceptible to several fungal pathogens causing enormous losses to the crop. Among all soil-borne fungal pathogens, *Phytophthora* and *Fusarium* cause the most severe damage to plants either in nurseries or orchards, citrus grooves affected with root rot pathogens cause 22% losses annually (Murara, 1988). Although many soil-borne pathogens are associated with citrus root rot complex, the most prominent are *Phytophthora* spp. (Timmer and Mange, 1988) and *Fusarium* spp. (Smith *et al.*, 1989). The objective of this study was to assess the effectiveness of fungicides as soil drenching and root dipping to control *Phytophthora* and *Fusarium* spp. attacking citrus rootstock at the seedling stage. Comparison of several citrus rootstocks in the greenhouse and field demonstrated that infection of young roots occurs very rapidly and at the same rate on susceptible and tolerant hosts,

but that root rot damage and fungal reproduction are limited subsequently on tolerant hosts (Graham, 1995). Control begins with the production of disease-free seedlings since much of the inoculums originate from an infested nursery. There are specific cultivars tolerant to some fungal pathogens in some cases, but the choice of cultivar depends upon the market's demand; thus, it is necessary to control disease on susceptible cultivars. Foot rot caused by *P. parasitica* can be effectively controlled with the proper application of certain fungicides followed by good management practices (Timmer and Castle, 1985).

Citrus rootstocks differ in their tolerance to soil salinity, cold injury and diseases. Rough lemon (*Citrus jambhiri*) rootstock is one of the rootstocks used for early maturity and large fruit size and to produce large healthy trees. Also, the trees grafted on Rough lemon are tolerant to drought, salinity, citrus tristeza virus and exocortis however it is sensitive to fungal diseases and should be used with proper care at every stage right from nursery to the orchard.

Conclusion: Plants soil drenched and root dipped with fungicides have maximum root shoot ratio, fresh dry weight ratio, photosynthetic rate, stomatal conductance and transpiration rate as compared to the untreated plants. Moreover, fungicides suppressed fungal growth.

Authors Contributions statement: SY conducted and analyzed the research and wrote the manuscript for M.Sc degree. MMK designed analyzed and supervised the entire study. AH conducted and analyzed the research and wrote the manuscript for her Ph.D degree. MA, BS, SA and MA helped in analyzing the data and research trial.

Conflict of interest: The authors have declared no conflicts of interest for this article.

Acknowledgement: The authors thanks to the Department of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan.

REFERENCES

- Ahmad, N., M.J. Jaskani, M.M. Khan, M. Abbas, A. Rehman and I. A. Khan. 2010. Screening of different citrus rootstocks against *Phytophthora* and *Fusarium* spp. Caderno de Pesquisa, Série Biologia. 22:75-88.
- Anonymous. 2019. Review on Pakistan's Export. Export Promotion Bureau of Statistics, Statistical division, Government of Pakistan, Islamabad.
- Bower, J.P. 1979. Water relations of *Phytophthora* infected Fuertes trees and their influence on management. South African avocado growers' association research report for. 3:25-27
- Einhellig, F.A. 1995. Allelopathy: Organisms, Process and Applications. American Chemical Society. Washington.pp. 96-116.
- El-Nouby, A.S.M. 2002. Citrus nematode problems, new approaches for its control in newly reclaimed lands. M.Sc. Thesis Fac. Agric. Cairo Univ. Egypt. 101.
- Fenn, M. and M.D. Coffey. 1984. Studies on in vitro and in vivo antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology. 74:606-611.
- Fleischmann, F., J. D. Schneider, R. Matyssek and W. F. Obwald. 2002. Investigations on net CO₂ assimilation, transpiration and root growth of *Fagus sylvatica* infested with four different *Phytophthora* species. Plant biol. 4:144-152.
- Gopi, R., C.A. Jaleel and R. Panneerselvam. 2008. Leaf anatomical responses of *Amorphophallus campanulatus* to triazoles fungicides. Eur Asia J Bio Sci. 2:46-52.
- Graham, J.H. 1995. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. Phytopathology. 85:111-117.
- Gundy, S.D.V. and P.H. Tsao. 1963. Growth reduction of citrus seedlings by *Fusarium solanias* influenced by the citrus nematode and other soil factors. Phytopathology. 53:488-489.
- Hussain, F. and Abid, M., 2011. Pest and diseases of chilli crop in Pakistan: A review. Int. J. Biol. Biotech. 8:325-332.
- Ippolito, A., L. Schena, F. Nigro, V.S. Ligorio and T. Yaseen. 2004. Real-time detection of *Phytophthora nicotianae* and *P. citrophthora* in citrus roots and soil. Eur. J. Plant Pathol. 110:833-843.
- Jaleel, C.A., P. Manivannan, B. Sankar, A. Kishorekumar, S. Sankari and R. Panneerselvam. 2007. Paclobutrazol enhances photosynthesis and ajmalicine production in *Catharanthus roseus*. Process Biochemistry. 42:1566-1570.
- Landis, T.D., R. W. Tinus, S. E. MacDonald and J. P. Barnett. 1990. The container tree nursery manual. Vol. 5, The biological component. Nursery pests and mycorrhizae, US Dep. Agric. For. Serv., Washington, DC. Agric. Handb. 674.
- Le Roux, H. F., Ware, A.B., Pretorius, M. C and Wenner, F. C. 1998. Comparative efficacy of pre plant fumigation and post plant chemical treatment of replant citrus trees in an orchard with *Tylenchulus semipenetrans*. Plant Disease. 82: 1323-1327.
- Marais, P.G and M. J. Hattingh. 1986. Reduction of root rot caused by *Phytophthora cinnamomi* in Grapevines by chemical treatment. Plant Disease. 70:109-111.
- Maurel, M., C. R. G. Capron and M. L. Desprez-Loustau. 2001. Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulation, mineral nutrition and vulnerability to

- water deficit of five oak and chestnut species. Forest Pathology. 31:353-369.
- Murara, P.R. 1988. Estimated average annual percent trees loss for Florida citrus industry. Proc. Fla. State Hort. Soc. 101:63-66.
- Nemec S., A. D. Stamper and L. G. Albrigo. 1986. Microscopy of *Fusarium solani* infected rough lemon citrus fibrous roots. Canadian Journal of Botany 64:2840-2847.
- Ploetz, R. C. and B. Schaffer. 1992. Effects of flooding and *Phytophthora* root rot on net gas exchange of avocado in Dade County, Florida. Proc. of Second World Avocado Congress. pp. 111-117.
- Rajput N.A., M.A. Pathan, M.M. Jiskani, A.Q. Rajput and R.R. Arain. 2008. Pathogenicity and host range of *Fusarium solani* (Mart.) sacc. causing dieback of Shisham (*Dalbergia sissoo* Roxb.). Pak. J. Bot. 40:2631-2639.
- Reiger, M. 2006. Citrus: Lemon, Lime, Orange, Tangerine and Orange. Horticultural Crops Programme, University of Georgia. <http://www.uga.edu/fruit/citrus.html>.
- Savita, G.S.V. and A. Nagpal. 2012. Citrus diseases caused by *Phytophthora* species. GERF Bulletin of Biosciences. 3:18-27.
- Siddiqui., Z.S. and A. Zaman. 2006. Effects of Benlate systemic fungicide on seed germination, seedling growth, biomass and phenolic contents in two cultivars of maize L. Pak. J. Bot. 36:577-582.
- Smith, A. G.A.G. Muhvich., K. H. Muhvich and C. Wood. 1989. Fatal *Fusarium solani* infections in baby sharks. J. Med. Vet. Mycol. 27:83-91.
- Snyman, C. P and J. M. Kotzé. 1983. Evaluation of application techniques of four fungicides for the control of *Phytophthora* root rot on avocado seedlings. S Afr Avocado Growers' Ass Yrbk. pp. 79-81.
- Spina, S., V. Coco, A. Gentile, A. Catara and G. Cirvellari. 2008. Association of *Fusarium solani* with *rolABC* and wild type Troyer citrange. J. Plant Pathol. 90: 479-486.
- Timmer, L. W. 1982. Host range and host colonization, temperature effects, and dispersal of *Fusarium oxysporum* f. sp. *citri*. Phytophthology 72:698-702.
- Umami, M., L.M. Parker and S.K. Arndt. 2021. The impacts of drought stress and *Phytophthora cinnamomi* infection on short-term water relations in two year-old *Eucalyptus obliqua*. Forests. 12:109.
- Walker., G. E. 1987. *Phytophthora* root-rot of container-grown citrus as affected by foliar sprays and soil drenches of phosphorous and acetyl salicylic acids. Plant and soil 107:107-112.